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**Application Note:** *Screening Cytoactive Compounds to Determine Cell Cycle Arrest*

# Screening Cytoactive Compounds to Determine Cell Cycle Arrest Using the Guava® PCA™-96 System, a Microplate-Based Benchtop Cell Analysis System

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## ABSTRACT

The rate of cell division in an organism is a tightly regulated process associated with growth and differentiation. Generally, cells do not undergo division unless signaled to enter the active segments of the cell cycle, or unless the normal regulation mechanism is disrupted. When disrupted, it is important to identify the genetic basis for this disruption in order to develop therapies to preferentially target those cells with abnormalities. One screening method for potential therapeutic drugs, or for the effect of specific genes on cell cycle regulation, is to measure changes in cell cycle kinetics and DNA content using propidium iodide (PI). For a variety of reasons, not all drugs will exhibit the same effects on all cell types hence multiple cell lines should be screened. To make the study of cytoactive compounds more efficient, the cell cycle protocol was adapted for use on a novel 96-well microplate-based benchtop cell analysis system, the Guava PCA-96 system, allowing the analysis of large numbers of compounds in a timely fashion. A panel of anti-inflammatory, anti-neoplastic and anti-infective drugs was screened on suspension and adherent cells to observe and identify the effect of the drugs, if any, on the phases of the cell cycle. Greater than 20 drugs out of 80 tested arrested the two cell lines in one of the phases of the cell cycle. With accurate and unambiguous results from the Guava PCA-96 system, researchers can rapidly screen drug compounds and explore key pathways controlling the cell cycle.


## INTRODUCTION

A number of potential molecular targets for anti-cancer drug discovery have been identified in cell cycle control mechanisms. Many cell cycle regulators that control the correct entry and progression through the cell cycle are altered in tumors. In fact, most, if not all, human cancers show a deregulated control of G1 progression, a period when cells decide whether to start proliferation or to stay quiescent. Manipulation of these control mechanisms provides new avenues for the design of advanced therapeutic strategies against tumor development. Potent and selective small-molecule mediated inhibition of the cell's replication machinery remains a principal aim in the development of novel therapeutics and biological probes. Recent efforts have identified small molecules capable of arresting the cell cycle via specific interaction with a variety of intracellular protein targets.[1]

For cells to divide they must first duplicate their nuclear DNA. The different stages of the cell cycle can be discriminated by simply determining the amount of DNA in each cell using propidium iodide (PI) staining. Resting cells (G0/G1 phase) contain two copies of each chromosome. As cells progress toward mitosis, they synthesize DNA (S phase), allowing more PI intercalation with an accompanying increase in fluorescence intensity. When all chromosomes have replicated and the DNA content has doubled (G2/M phase), the cells fluoresce with twice the intensity of the G0/G1 popula-

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tion. The G2/M cells eventually divide into two cells with the DNA equally distributed between the daughters, thus halving the intensity of the PI stain.

The Guava PCA-96 system with the optimal cell cycle protocol and reagents was used to screen for cell cycle arrest induced by 80 anti-inflammatory, anti-neoplastic and anti-infective compounds on both suspension and adherent cell lines in a 96-well format. Cells were treated with test compounds and controls, fixed, permeabilized and stained with PI according to the Guava PCA-96 Cell Cycle Protocol. Data from the stained cells were acquired on the Guava PCA-96 system using CytoSoft software and analyzed using ModFit LT™, a third party curve fitting software. A number of compounds were easily and quickly shown to arrest cells in one of the phases of the cell cycle.

## MATERIALS AND METHODS

### *Reagent Preparation*

Eighty cytotoxic agents, anti-proliferatives, immune suppressants, anti-infectives or other experimental and therapeutic agents were obtained from MicroSource Discovery Systems in a 96-well format and were reconstituted to 10 mM with DMSO. The compounds were then adjusted to a stock concentration of 2 mM with DMSO. On the day of drug induction, the compounds were diluted to a working stock of 40 µM in media. The final compound concentration mixed with the cells was 10 µM for both cell lines used. Negative controls (media only, n=8), vehicle controls (DMSO diluted at final dilution of 1:100 in media, n=2) and positive controls (aphidicolin, nocodazole, or mevinolin; n=3) were also assayed in the same plates on the same days.

### *Suspension Cell Line*

Jurkat cells (ATCC# Cat. No. TIB-152), derived from a patient with T cell leukemia, were grown in suspension and split 1:3 with complete medium [RPMI 1640 containing sodium pyruvate, D-glucose, L-glutamine and 10% fetal bovine serum (FBS)]. The next day, cells were synchronized by serum starvation in the complete medium but without FBS for 24 hours in a T75 flask. After serum starvation, 100,000 cells in 150 µL were seeded in each well of a 96-well flat bottom tissue culture plate. A volume of 50 µL of 40 µM working stock of each compound or control (50 ng/mL aphilicolin or 29 ng/mL nocodazole, final concentration) was added to each well containing the cells and incubated for 24 hours in a 37 °C incubator containing 5% $CO_2$. After incubation, the cells were fixed overnight, stained and acquired according to the Guava PCA-96 Cell Cycle Protocol.

### *Adherent Cell Line*

PC3 cells (adherent human prostate cancer cells) were seeded overnight at 100,000 cells in 500 µL in 24-well flat bottom plates with F12K media containing L-glutamine and 10% FBS. Cells were cultured overnight and then serum starved in F12K complete medium without FBS for 30 hours for synchronization. Cells were induced with drugs at final concentration of 10 µM or controls (30 µg/mL mevinolin or 29 ng/mL nocodazole, final concentration) for 24 hours in a 37 °C incubator with 5% $CO_2$. After incubation, cells were trypsinized from the plate and transferred to a 96-well round bottom plate. Cells were fixed overnight, stained and acquired the next day according to the Guava PCA-96 Cell Cycle Protocol.

## INSTRUMENT SETTINGS AND DATA ANALYSIS

The cells were acquired on the Guava PCA-96 system collecting 2500 events using the very low flow rate. Data were analyzed with ModFit LT (Verity) with the auto-aggregation and auto-debris features turned on to determine the percentages of cells in the G0/G1, S and G2/M phases of the cell cycle. A compound was considered to have induced cell cycle arrest if the percentages of cells in one of the phases of the cell cycle were 3 SD above the average of the negative and vehicle controls.

## RESULTS

Not all drugs will exhibit the same effects on all cell types due to differences in susceptibility of available genetic targets and differences in transporter molecules among other reasons. In previous work, we demonstrated that the drugs aphidicolin (S phase arrest) and nocodazole (G2/M phase arrest) specifically arrested Jurkat cells in the expected phases of the cell cycle compared to untreated controls, while mevinolin (G0/G1 and G2/M phase arrest) showed no effect. In contrast, with the prostate cancer cell line PC3, mevinolin and nocodazole demonstrated the expected cell cycle arrest, while aphidicolin showed no effect. Thus, for this study, we screened all compounds against

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both cell lines and as expected found compounds with activity against both or only one or the other.

Typically, when released from serum starvation and cultured for an additional 24 hours, the Jurkat cells were primarily in the G0/G1 and S phases while the PC3 cells were in the G0/G1 and G2/M phases. Treatment with compounds which arrest the cells resulted in changes in the percentage of cells in each phase relative to the cells incubated in the absence of compound.

Figure 1 shows Jurkat cells arrested at certain phases of the cell cycle compared to negative control. The negative control (Fig. 1A) had the usual percentage of cells in the G0/G1, S and G2/M phases, while Jurkat cells treated with representative compounds from the panel of 80 drugs screened arrested in G0/G1, S and G2/M, respectively (Fig. 1B, 1C, and 1D) did not. When cells are arrested, one phase is enriched and the plot shows a broader and/or taller peak for that particular phase. Jurkat cells incubated with medium only (negative control) showed 43% of cells in the G0/G1 phase, 38% in S phase and 13% in G2/M phase (Fig. 1A). Jurkat cells treated with methotrexate showed a G0/G1 phase arrest with 59% of the cells in G0/G1 compared to the control value of 49% (Fig. 1B). Jurkat cells treated with lefunamide showed an S phase arrest with 59% of the cells in S compared to the control value of 38% (Fig. 1C). Jurkat cells treated with podophyllotoxin showed a G2/M phase arrest with 80% of the cells in G2/M compared to the control value of 13% (Fig. 1D). Figure 2 shows representative plots of PC3 cells that were serum starved for 30 hours and then treated with drugs for 24 hours. PC3 cells incubated with medium only (negative control) showed 47% of cells in the G0/G1 phase, 16% in S phase and 37% in G2/M phase (Fig. 2A). PC3 cells treated with emetine-HCl showed a G0/G1 phase arrest with 60% of the cells in G0/G1 compared to the control value of 47% (Fig. 2B). PC3 cells treated with mechlorethamine showed an S phase arrest with 58% of the cells in S compared to the control value of 16% (Fig. 2C). PC3 cells treated with albendazole showed a G2/M phase arrest with 55% of the cells in G2/M compared to the control value of 37% (Fig. 2D).

Results obtained for the 80 compounds cultured with Jurkat and PC3 cells are summarized below. If the cell concentration was less than 2 SD of the average

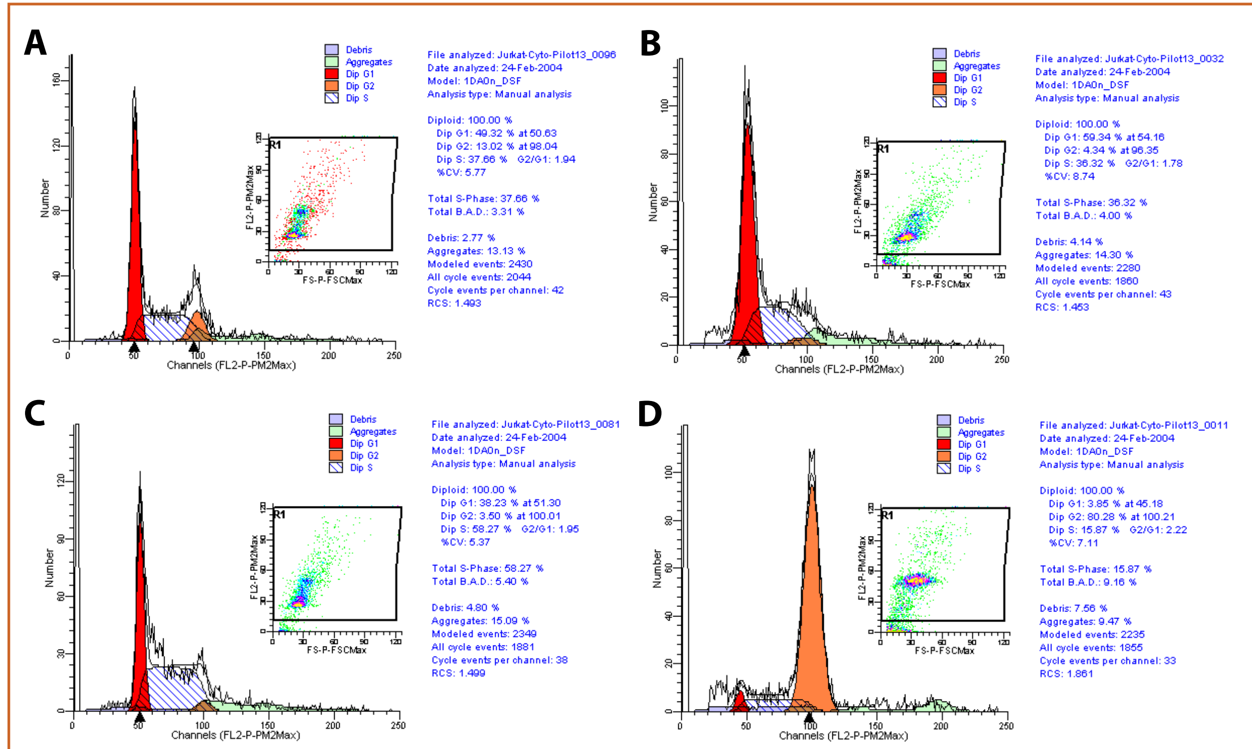


FIGURE 1: Jurkat cells were incubated with: A) medium only (negative control) ; B) treated with methotrexate ; C) lefunamide; or D) podophyllotoxin for 24 hours after synchronization. Representative plots are shown here.

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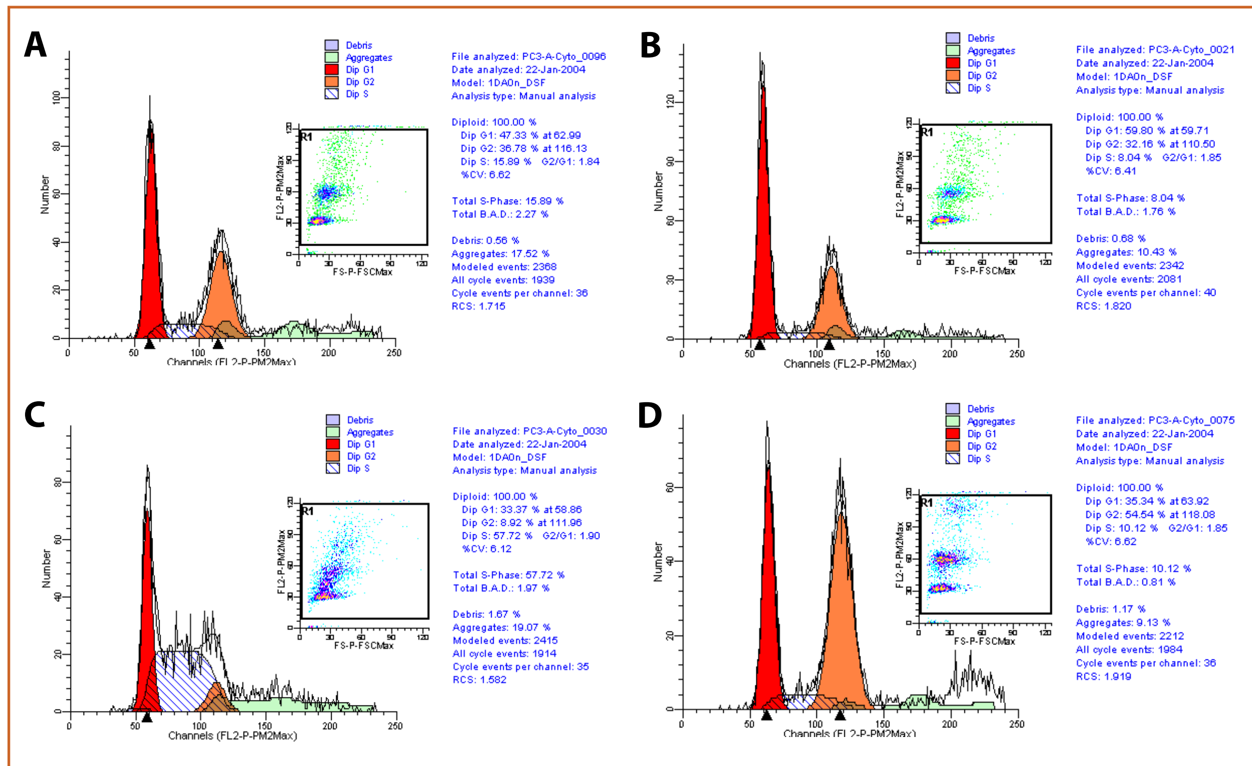


FIGURE 2: PC3 cells were incubated with: A) medium only (negative control); B) treated with emetine HCl; C) mechlorethamine; or D) albendazole for 24 hours after synchronization. Representative plots are shown here.

control, the compound was considered a potent drug, causing cell necrosis or apoptosis. If the cells also showed a very broad G0/G1 phase, the compound was considered a strong G0/G1 phase arrestor. For compounds which had no significant affect on cell concentration, a compound was considered a 'hit' if the results showed a difference greater than 3 SD compared to the average of the negative (media only) controls. Results showed that DMSO at a 1:100 dilution final concentration did not have any affect on cell cycle for either cell line. In each plate, two positive controls were used at known concentrations to arrest cells in the expected phase of the cell cycle.

Table 1 shows the results of six different plates of Jurkat cells assayed with the 80 compounds from the cancer plate plus the controls. The plates were assayed on either a different day (n=4) or on the same day but on different instruments (n=2).

While the negative and positive controls showed the expected results in every assay, with the positive controls showing the correct cell cycle phase arrest in all experiments, in some cases, the compounds showed multiple effects when assayed on different plates or different days. Thus, we could not determine what consistent effect, if any, those compounds were having on cycling cells. Other compounds had no effect at any time. Twenty-one of the 80 compounds did consistently arrest Jurkat cells in one phase of the cell cycle. Twelve compounds arrested cells in G0/G1 phase, three in S phase and six in G2/M phase. For example, podophyllotoxin, mercaptopurine and mycophenolic acid showed consistent G2/M, S and G0/G1 phase arrest, respectively. Five of the compounds caused massive cell loss either through necrosis or apoptosis.

Table 2 shows the results of four different plates of PC3 cells treated with the 80 compounds plus controls. The plates were assayed on either a different day (n=2) or on the same day but on different instruments (n=2).

Most (21) of the 'hits' observed with the cancer plate compounds were for G0/G1 phase arrest for PC3 cells. There was only one compound (mechlorethamine) that showed S phase arrest in PC3 cells. There were five compounds that showed G2/M arrest (podophyllotoxin, colchicine, mebendazole, fenbendazole, and albendazole).

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## Table 1. Summary of Jurkat Cell Screening

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Neg Control | Solasodine | Aklavine HCl | Juglone | Celastrol | Usnic Acid |
| B | Neg Control | Cyclophosphamide Hydrate | Chlorambucil | Diethylcarbamazine Citrate | Chloramphenicol Hemisuccinate | Cloroquine |
| C | Neg Control | Hydroxyurea | Nitromide | Nitrofurazone | Vidarabine | Mechlorethamine |
| D | DMSO | Berberine Chloride | Mycophenolic Acid | Quinacrine HCl | Pyrimethamine | Mebendazole |
| E | DMSO | 1,2-Dimethyl Hydrazine HCl | Fenbendazole | 5-Fluoro-5'-Deoxyuridine | Amygdalin | Zidovudine |
| F | Aphilicolin Pos Control | Azaserine | Dinitolmide | Semustine | Parthenolide | Cycloheximide |
| G | Aphilicolin Pos Control | Urethane | Albendazole | 3-Aminobenzamide | Conessine | Busulfan |
| H | Aphilicolin Pos Control | Acridone | Camptothecin | Cinchonine | Altretamine | Thiabendazole |

| | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| A | Helenine | Sanguinarine Sulfate | Amprolium | Azathioprine | Podophyllotoxin | Nocodazole Pos Control |
| B | Colchicine | Mercaptopurine | Emetine HCl | Fluorouracil | Thioguanine | Nocodazole Pos Control |
| C | Trioxsalen | Methotrexate | Methoxsalen | Metronidazole | Primaquine Diphosphate | Nocodazole Pos Control |
| D | Streptozosin | Tobramycin Sulfate | Tamoxifen Citrate | Acriflavinium HCl | Ellagic Acid | Neg Control |
| E | 5-Azacytidine | Foscarnet | Emodin | Carboplatin | *p*-Fluorophenyl-alanine | Neg Control |
| F | Floxuridine | Edoxudine | Cisplatin | Carmustine | Mitotane | Neg Control |
| G | Monocrotaline | Aklomide | Lefunamide | Methoxamine HCl | Diazaquone | Neg Control |
| H | Nicolsamide | Chloramphenicol | Hexestrol | Rotenone | Formestane | Neg Control |

LEGEND:

| G0/G1 phase | S phase | G2/M phase | Low cell conc. | Cannot determine | No effect |
|---|---|---|---|---|---|

Table 3 shows a summary of the effects of the compounds on the cell cycle for Jurkat and PC3 cells. It also shows the clinical use and mechanisms of actions for the some of the compounds used in this study. Some compounds, such as lefunamide, showed S phase arrest on Jurkat cells but had no effect on PC3 cells. Some compounds such as podophyllotoxin showed G2/M phase arrest in both Jurkat and PC3 cells. The "bendazole" compounds causes G2/M phase arrest in PC3 similar to Jurkat cells. Compounds such as aklavine HCl and camptothecin show a very broad G0/G1 peak around the subzero region indicating that they have apoptosis inhibition and strong effects of G0/G1 cell cycle phase arrest.

## DISCUSSION

The Guava PCA-96 system with the optimal cell cycle reagents and sample preparation protocol is capable of screening compounds for cell cycle phase arrest in both suspension and adherent cell lines in a 96-well format.

Of the 80 compounds screened, a few compounds induced massive cell necrosis or apoptosis resulting in low cell concentrations. Among these were celastrol, campothecin, rotenone, sanguinarine sulfate and chloramphenicol when assayed with Jurkat cells. Only celastrol and campothecin showed such activity towards the PC3 prostate cancer cells. In some of these cases, the histogram of DNA content showed a sub-G0/G1 population and/or very broad peaks of G0/G1 suggesting the cells have induced apoptosis and/or death. In fact, camptothecin, which showed

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## Table 2. Summary of PC3 Cell Screening

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Neg Control | Solasodine | Aklavine HCl | Juglone | Celastrol | Usnic Acid |
| B | Neg Control | Cyclophosphamide Hydrate | Chlorambucil | Diethylcarbamazine Citrate | Chloramphenicol Hemisuccinate | Cloroquine |
| C | Neg Control | Hydroxyurea | Nitromide | Nitrofurazone | Vidarabine | Mechlorethamine |
| D | DMSO | Berberine Chloride | Mycophenolic Acid | Quinacrine HCl | Pyrimethamine | Mebendazole |
| E | DMSO | 1,2-Dimethyl Hydrazine HCl | Fenbendazole | 5-Fluoro-5'-Deoxyuridine | Amygdalin | Zidovudine |
| F | Mevinolin Pos Control | Azaserine | Dinitolmide | Semustine | Parthenolide | Cycloheximide |
| G | Mevinolin Pos Control | Urethane | Albendazole | 3-Aminobenzamide | Conessine | Busulfan |
| H | Mevinolin Pos Control | Acridone | Camptothecin | Cinchonine | Altretamine | Thiabendazole |

| | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| A | Helenine | Sanguinarine Sulfate | Amprolium | Azathioprine | Podophyllotoxin | Nocodazole Pos Control |
| B | Colchicine | Mercaptopurine | Emetine HCl | Fluorouracil | Thioguanine | Nocodazole Pos Control |
| C | Trioxsalen | Methotrexate | Methoxsalen | Metronidazole | Primaquine Diphosphate | Nocodazole Pos Control |
| D | Streptozosin | Tobramycin Sulfate | Tamoxifen Citrate | Acriflavinium HCl | Ellagic Acid | Neg Control |
| E | 5-Azacytidine | Foscarnet | Emodin | Carboplatin | *p*-Fluorophenyl-alanine | Neg Control |
| F | Floxuridine | Edoxudine | Cisplatin | Carmustine | Mitotane | Neg Control |
| G | Monocrotaline | Aklomide | Lefunamide | Methoxamine HCl | Diazaquone | Neg Control |
| H | Nicolsamide | Chloramphenicol | Hexestrol | Rotenone | Formestane | Neg Control |

LEGEND:

| G0/G1 phase | S phase | G2/M phase | Low cell conc. | Cannot determine | No effect |
|---|---|---|---|---|---|

sub-G0/G1 events and a broad G0/G1 peak, is a well-known apoptosis inducer. Moreover, rotenone and sanguinarine sulfate, though not chloramphenicol, were shown to induce apoptosis in Jurkat cells when screened using the Guava Nexin® assay which detects both early and late apoptosis.[2] Thus, the Guava cell cycle assay can also detect the induction of apoptosis in at least some instances. In contrast, celastrol, which only showed a low cell concentration, had been shown to dissolve the plasma membrane, leaving only nuclei intact.[2]

Some of the compounds screened, such as colchicine, methotrexate, hydroxyurea and cycloheximide, are known to cause cell cycle arrest and did show activity against one or both cell lines. Others showed the expected cell cycle arrest based on their mechanism of action. For example, mechlorethamine, which induced S phase arrest in PC3 cells, is known to crosslink DNA.

In addition, the four of five compounds which showed G2/M arrest in Jurkat and PC3 cells (podophyllotoxin, mebendazole, fenbendazole and albendazole) are all known inhibitors of microtubules, which are necessary for the cell to partition the duplicated chromosomes into the daughters.

Three of the microtubule disruptors, mebendazole, fenbendazole and albedazole, each structurally related to the others, were shown to arrest cells in G2/M. Interestingly, a fourth member of this family, thiabendazole, had no such activity. This result is identical to the one obtained when these four compounds were screened using the Guava ViaCount® and Guava Nexin assays for apoptotic or cytotoxic activity against Jurkat cells. That is, only thiabendazole did not induce apoptosis. One report showed that thiabendazole apparently has a different mechanism of action from the

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## Table 3. Overall Summary of Results

| Well No. | Well No. | Compounds | Jurkat | PC3 | Clincal Indication | Mechanism of Action |
|---|---|---|---|---|---|---|
| 2 | A02 | **Solasodine** | no effect | no effect | antineoplastic, antinflammatory | |
| 3 | A03 | **Aklavine HCl** | | | antibiotic, antineoplastic | |
| 4 | A04 | **Juglone** | no effect | | antineoplastic, antifungal | inhibitor of peptidyl-prolyl *cis/trans* isomerases |
| 5 | A05 | **Celastrol** | | | antineoplastic, anti-inflammatory | inhibits pro-inflammatory cytokine release |
| 6 | A06 | **Usnic Acid** | | no effect | antibiotic | |
| 7 | A07 | **Helenine** | | | antineoplastic, antibacterial, anthelmintic | |
| 8 | A08 | **Sanguinarine Sulfate** | | | antineoplastic | |
| 9 | A09 | **Amprolium** | | | coccidiostatic | |
| 10 | A10 | **Azathioprine** | | no effect | immunosuppressive, antineoplastic | inhibits DNA replication |
| 11 | A11 | **Podophyllotoxin** | | | antineoplastic | inhibits uT assembly, M arrest |
| 14 | B02 | **Cyclophosphamide Hydrate** | no effect | no effect | antineoplastic | crosslinks DNA |
| 15 | B03 | **Chlorambucil** | | no effect | antineoplastic | crosslinks DNA |
| 16 | B04 | **Diethylcarbamazine Citrate** | no effect | | Anthelminic | |
| 17 | B05 | **Chloramphenicol Hemisuccinate** | | | antibacterial, antirickettsial | inhibits protein synthesis |
| 18 | B06 | **Cloroquine** | no effect | | antimitotic, antigout agent | |
| 19 | B07 | **Colchicine** | | | gout suppressant | inhibits uT assembly, spindle poison |
| 20 | B08 | **Mercaptopurine** | | no effect | antineoplastic | inhibits DNA replication; pur antimetabolite |
| 21 | B09 | **Emetine HCl** | | | antiamebic | inhibits RNA synthesis |
| 22 | B10 | **Fluorouracil** | | no effect | antineoplastic | inhibits nucleic acid, replication, tetratogen inhibits thymilidate synthetase |
| 23 | B11 | **Thioguanine** | | | antineoplastic | inhibits DNA replication; pur antimetabolite |
| 26 | C02 | **Hydroxyurea** | no effect | | antineoplastic | inhibits ribonucleoside diphoshate reductase (S-phase/G-1 inhibitor) |
| 27 | C03 | **Nitromide** | | | antibacterial; coccidiostat | |
| 28 | C04 | **Nitrofurazone** | no effect | | topical anti-infective | |
| 29 | C05 | **Vidarabine** | | no effect | antiviral | |
| 30 | C06 | **Mechlorethamine** | | | antineoplastic | crosslinks DNA |
| 31 | C07 | **Trioxsalen** | no effect | no effect | melanizing agent, antipsoriatic | |
| 32 | C08 | **Methotrexate** | | | immunosuppressive, antineoplastic | folic acid antagonist |
| 33 | C09 | **Methoxsalen** | no effect | no effect | antipsoriaticm | pigmentation agent |
| 34 | C10 | **Metronidazole** | no effect | no effect | antiprotozoal | |
| 35 | C11 | **Primaquine Diphosphate** | | | antimalarial | |
| 38 | D02 | **Berberine Chloride** | no effect | | antipyretic, antimalarial | |
| 39 | D03 | **Mycophenolic Acid** | | | antineoplastic | inhibits purine synthesis |
| 40 | D04 | **Quinacrine HCl** | | | anthelmintic | phospholipase inhibitor |
| 41 | D05 | **Pyrimethamine** | | | antimalarial | inhibits dihydrofolate reductase-thymidylate synthase (DHFR-TS). |
| 42 | D06 | **Mebendazole** | | | anthelmintic | inhibits tubulin polymerization |
| 43 | D07 | **Streptozosin** | no effect | no effect | antineoplastic | alkylating agent binds to DNA |
| 44 | D08 | **Tobramycin Sulfate** | no effect | | antibacterial, antirickettsial | inhibits protein synthesis |
| 45 | D09 | **Tamoxifen Citrate** | no effect | | anti-cancer | estrogen antagonist |
| 46 | D10 | **Acriflavinium HCl** | | | anti-infective | DNA intercalator |
| 47 | D11 | **Ellagic Acid** | | | antineoplastic | inhibits DNA topoisomerases |
| 50 | E02 | **1,2-Dimethyl-Hydrazine HCl** | no effect | | antineoplastic | generate methyl carbonium ions |
| 51 | E03 | **Fenbendazole** | | | anthelmintic | inhibits tubulin polymerization |

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## Table 3 (continued). Overall Summary of Results

| Well No. | Well No. | Compounds | Jurkat | PC3 | Clincal Indication | Mechanism of Action |
|---|---|---|---|---|---|---|
| 52 | E04 | **5-Fluoro-5'Deoxyuridine** | | | antineoplastic | inhibits DNA replication; pur antimetabolite |
| 53 | E05 | **Amygdalin** | no effect | no effect | anti-inflammation, experimental antineoplastic | |
| 54 | E06 | **Zidovudine** | no effect | no effect | antiviral | RT transferase inhibitor |
| 55 | E07 | **5-Azacytidine** | | no effect | antineoplastic | inhibits DNA replication; cytosine analog |
| 56 | E08 | **Foscarnet** | no effect | no effect | antiviral | |
| 57 | E09 | **Emodin** | no effect | no effect | antimicrobial, antineoplastic, cathartic | |
| 58 | E10 | **Carboplatin** | no effect | no effect | antineoplastic | crosslinks DNA |
| 59 | E11 | **p-Fluorophenyl Alanine** | | no effect | aminoacid antagonist | protein synthesis inhibitor |
| 62 | F02 | **Azaserine** | | no effect | antineoplastic | amino acid antagonist |
| 63 | F03 | **Dinitolmide** | no effect | no effect | antiprotozoal | |
| 64 | F04 | **Semustine** | no effect | no effect | antineoplastic | |
| 65 | F05 | **Parthenolide** | no effect | | anti-inflamation | immune suppressant |
| 66 | F06 | **Cycloheximide** | | | | inhibits protein synthesis |
| 67 | F07 | **Floxuridine** | | | antineoplastic | antimetabolite |
| 68 | F08 | **Edoxudine** | | | antiviral | |
| 69 | F09 | **Cisplatin** | no effect | no effect | antineoplastic | crosslinks DNA |
| 70 | F10 | **Carmustine** | | no effect | antineoplastic | |
| 71 | F11 | **Mitotane** | | no effect | antineoplastic, insecticide | |
| 74 | G02 | **Urethane** | | no effect | antineoplastic, cytotoxic | |
| 75 | G03 | **Albendazole** | | | anthelmintic | inhibits tubulin polymerization |
| 76 | G04 | **3-Aminobenzamide** | no effect | no effect | antiproliferative | killer cell blocker |
| 77 | G05 | **Conessine** | no effect | | | |
| 78 | G06 | **Busulfan** | | no effect | antineoplastic | crosslinks guanine residues |
| 79 | G07 | **Monocrotaline** | no effect | no effect | antineoplastic | |
| 80 | G08 | **Aklomide** | no effect | | antiprotosoal | |
| 81 | G09 | **Lefunamide** | | no effect | antineoplastic | PDGF receptor blocker |
| 82 | G10 | **Methoxamine HCl** | | | mutagen | blocks DNA replar hydroxy-methyltransferase inhibitor |
| 83 | G11 | **Diazaquone** | | | antineoplastic | |
| 86 | H02 | **Acridone** | | no effect | | |
| 87 | H03 | **Camptothecin** | | | antineoplastic | inhibits DNA topoisomerase I |
| 88 | H04 | **Cinchonine** | | no effect | antimalarial | |
| 89 | H05 | **Altretamine** | no effect | no effect | antineoplastic | |
| 90 | H06 | **Thiabendazole** | no effect | no effect | anthelmintic | |
| 91 | H07 | **Nicolsamide** | | | anthelmintic | |
| 92 | H08 | **Chloramphenicol** | | | antibacterial | inhibits protein synthesis |
| 93 | H09 | **Hexestrol** | no effect | no effect | antineoplastic | (estrogen) |
| 94 | H10 | **Rotenone** | | | antineoplastic | mitochondrial poison |
| 95 | H11 | **Formestane** | | | antineoplastic | aromatase inhibitor |

LEGEND:

| G0/G1 phase | S phase | G2/M phase | Low cell conc. | Cannot determine | No effect |
|---|---|---|---|---|---|

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other three and hence might not be expected to affect cells in a similar manner as other family members.[2,3]

Some of the compounds showed different results when tested on the same cell line on different days so no consistent effect on the cell cycle was determined. In some cases, this was probably due to the degradation and oxidation of the compound during multiple freeze-thaw cycles or due to poor storage conditions. The compounds were stored at –20 °C instead of –80 °C. It is also possible that some compounds were rather weak inhibitors and so on some days did not appear potent enough to be scored as a 'hit'. Finally, while in most cases the SD were acceptably low, it is possible that the cut-off of greater than 3 SD above the average of the negative control was not sufficiently high enough to identify only robust 'hits'. For example, while the average of the cells in the G0/G1 phase on untreated Jurkat was 45.5% with standard deviation of 2.0, cells treated with thioguanine had 53.0% of cells in the G0/G1 phase, barely 3 SD above the negative control. Results obtained with other compounds, such as cyclophosphamide hydrate and cisplatin which showed no effect, were probably due to low concentration of the compounds used in this study.

Not all compounds will be active against all cell types. However, during the screening of the 80 compounds, if the compound exhibited an effect on both Jurkat and PC3 cells, it arrested both cells in the same cell cycle phase. Some compounds arrested Jurkat cells in one cell cycle phase (azathioprine and mercaptopurine for G0/G1) but showed no effect on PC3 cells and vice versa. The best agreement between cell lines was for compounds which arrest cells in G2/M, with 4 of the 7 active compounds effective on both cell lines. For S phase-arresting compounds, none of the 4 'hits' were active against both cell lines. Only 6 of the 37 compounds which arrested cells in G0/G1 were active against both cell lines. In these cases, the compound may not be at a high concentration enough to show cell cycle arrest in the both cells, or one cell line may have been more susceptible to arrest by that compound than the other line.

## SUMMARY AND CONCLUSION

The Guava PCA-96 system with the optimal protocol and reagents can be used to conveniently screen for compounds which induce cell cycle arrest. In order to determine accurately whether a compound has induced cell cycle arrest, duplicate plates should be assayed on multiple days. Some compounds are highly sensitive to degradation or oxidation due to freeze/thaw cycles and sub-optimal storage conditions. Correct storage and handling of the compounds is important to generate reproducible results. The cancer plate contains numerous compounds that arrested Jurkat and PC3 cells in G0/G1, S and G2/M phases. The Guava PCA-96 system can distinguish highly potent compounds which induced cell loss through apoptosis or necrosis and subsequently appear to arrest cells in the G0/G1 phase in a convenient 96-well format. The Guava PCA-96 system can accurately screen cytoactive and cytostatic compounds rapidly and effectively.

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